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Minireview

RNase MRP/RNase P: a structure–function relation conserved in evolution?

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RNase P and RNase MRP are related ribonucleoproteins. RNase MRP processes mitochondrial precursor- (primer) RNAs, whereas RNase P cleaves precursor-tRNAs to produce their mature 5′-ends. Both RNase P and RNase MRP are associated with the Th/To ribonucleoprotein suggesting possible interrelated pathways and/or functions. All known RNase P and RNase MRP RNAs contain conserved structural elements possibly involved in catalysis/substrate binding, but these elements do not predict all cellular functions of the RNPs.

Autoantibody; DNA replication; Mitochondrion; RNase P; tRNA precursor

1. INTRODUCTION

Ribonuclease mitochondrial RNA processing (RNase MRP) cleaves model substrates such as precursor-(primer) RNAs from the mammalian mitochondrial displacement-loop containing region (D-loop mtRNA) [1–4]. Ribonuclease P (RNase P) is the cellular ribonucleoprotein (RNP) endoribonuclease that cleaves precursor-transfer RNAs (pre-tRNAs) to generate the mature 5′-termini of tRNAs [5]. Both RNase MRP and RNase P are RNP endoribonucleases; i.e. both RNA and protein components are required for the ribonuclease activity of these RNP enzymes.

RNase MRP was originally identified in a screen for activities potentially involved in mouse mitochondrial primer RNA processing [1–2a] and it turned out that the enzyme copurified with nucleus-encoded RNAs [6,7]. Upon sequence analysis of the human MRP RNA gene (coding for the major RNA molecule copurified with RNase MRP) its identity with the previously characterized small stable 7-2 RNA was established; in addition this sequence analysis showed that the MRP/7-2 RNA is related to H1 RNA (also known as 8-2 RNA) the major RNA molecule copurified with human RNase P [8–12]. RNase MRP was prepared from nuclear extracts [4]. No convincing evidence for a location of the full-length MRP RNA within the mitochondrial matrix was found [13]. Taken together these findings suggested the possible need for the standardization of the RNase P/MRP nomenclature (see [1–33], Fig. 1, Fig. 2 and section 3).

All known RNase MRP and RNase P RNAs contain a version of a short conserved sequence overlapping with a proposed pseudo-knot [14–28a] (Fig. 1). This is a long-range helical element that involves base-pairing of an RNA loop with sequences outside the loop [29]. The short conserved sequence (as well as the overlapping pseudo-knot, termed long-range helix 'cage' or 'C'), may be diagnostic for these RNAs [14–28a]. Because of its conservation in all known MRP and in RNase P RNAs helix C was suggested to be part of the catalytic center of these enzymes [14].

2. CELLULAR RELATION OF RNase P AND RNase MRP

The key cellular relation is the association of RNase P and RNase MRP with the Th/To RNP [4,9–12,20,31–33,49]. All Th/To RNP autoantibodies (as defined by the co-immunoprecipitation of the two RNAs and four major proteins including Th40, also known as 40 kDa To autoantigen [4,31–33]) quantitatively and specifically immunodeplete the RNase MRP and RNase P activity as isolated from mammalian nuclear cell extracts. A conserved stem-loop structure, implicated in RNA–protein interactions in vitro [32] and in a mammalian cellular RNP reconstitution system [33] is present in RNase P and in the known metazoan MRP RNAs [14–28a]. A 5-nucleotide stem ('t-stem') of this stem-loop structural element is conserved with regard

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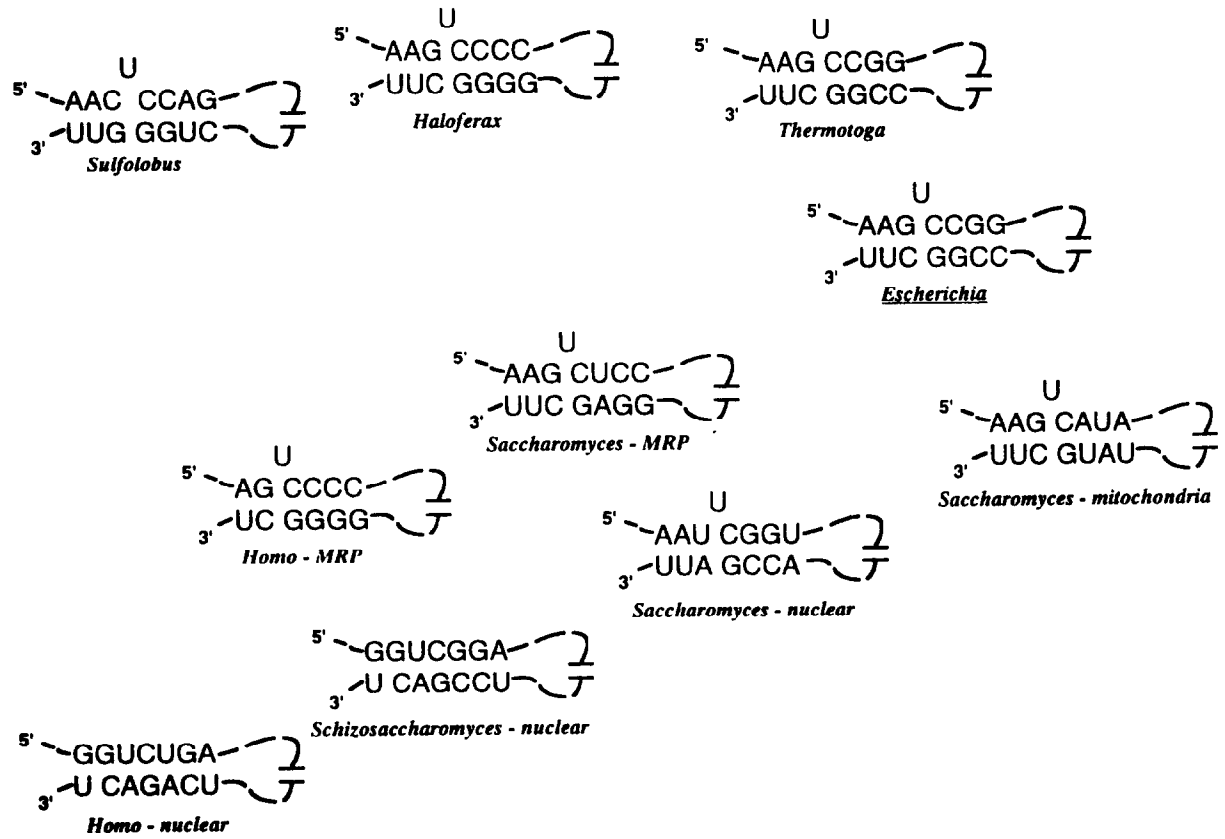

Fig. 1. Proposed conservation of long-range helix 'cage' = 'C' in all RNase P/MRP RNAs [14]. Examples of RNase P/MRP RNAs (the catalytic *E. coli* RNase P RNA is underlined) from all three domains [30,30a]: archaeal (*Haloferax*, *Sulfolobus*), distant procaryal (*Escherichia*, *Thermotoga*) as well as organellar (*Saccharomyces* mitochondria), and distant eucaryal (*Saccharomyces*, *Schizosaccharomyces*, Homo). The sequences are derived from the EMBL/GeneBank nucleic acid data banks and [15]. The helices are drawn 5′ to 3′ as suggested by N.C. Martin [16].

to the nucleotide bias as well as the approximate position in the known vertebrate RNase MRP RNAs, and in distinct bacterial RNase P RNAs, including the catalytic *E. coli* RNase P RNA (Fig. 2). Interestingly, the t-stem as well as base-pair opening in the stem are conserved in the bacterial RNase P RNA consensus structure and the stem has been implicated in the *E. coli* RNase P function [28,28a].

3. RNA PROCESSING BY RNase P, RNase MRP AND BY THE PROTOTYPE BACTERIAL CATALYTIC RNase P RNA

The most important domains for the recognition of pre-tRNAs by RNase P are contained in the acceptor stem and T arm, with the acceptor stem being a critical element for the selection of the precise cleavage site [34–41]. Eucaryal RNase P appears less flexible than bacterial RNase P and thus may require different features for pre-tRNA processing [34,39]: while bacterial RNase P (or M1 RNA, the catalytic RNA component of the *E. coli* RNase P [5,34]) cleaves minimal model substrates by means of a simple 'external guide sequence' (EGS, contained in the acceptor stem and T stem) [34–36] eucaryal RNase P enzymes do not cleave these minimal model substrates [34,36,39,41]. A more extensive 'guide sequence' (that contains several tRNA elements including the acceptor-stem, D-stem and T-stem) is required to target model RNA substrates for cleavage by human RNase P in vitro [41].

Mammalian RNase MRP enzymes cleave mitochondrial precursor-(primer) RNAs in a conserved manner [3,4,22] and the mouse mitochondrial model substrate D-loop mtRNA at multiple, discrete and interdependent sites [4]. One site, 'site 1' of D-loop mtRNA processing by RNase MRP, is at a RNA–DNA transition site possibly generated in the course of mitochondrial replication ([1–4,42,42a], but see Introduction). Deletion, replacement and point mutagenesis have indicated no primary sequence and a distance requirement for RNA processing by RNase MRP [4]. Substrate RNA folding may be effected by several of these mutations and therefore – similar to RNase P – the particular folding of the substrate RNA into a certain structure may be the predominant determinant for cleavage site selection by RNase MRP [5,38,40,43]. Experimental analysis of wildtype and mutant D-loop mtRNA folding and its influence on RNase MRP cleavage site selection will

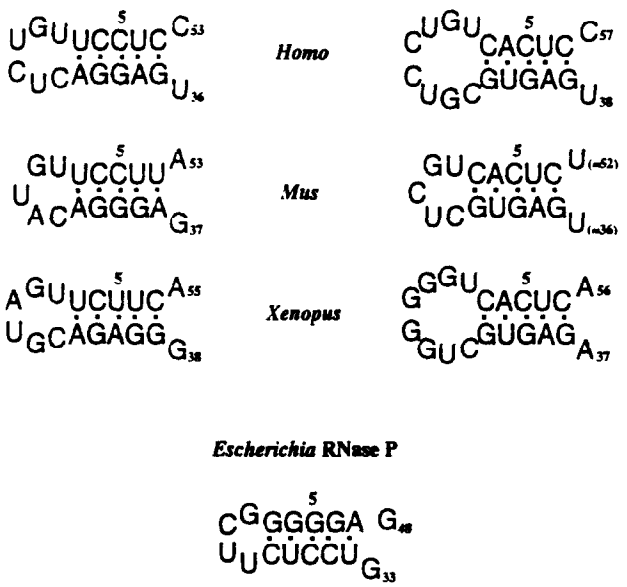

Fig. 2. Proposed conservation of the t-stem in distinct eucaryal RNase P/MRP RNAs and in the *E. coli* RNase P RNA. The sequences shown are derived from the DNA sequences: *E. coli* RNase P RNA [5]. Left column (from top to bottom): human KB cell MRP RNA [19]; mouse La9 cell MRP RNA [7]; *Xenopus laevis* MRP RNA [20]. Right column (from top to bottom): human HeLa cell H1 RNA copurified with RNase P [48]; mouse La9 cell RNA copurified with RNase P ([4]; Rossmanith and Karwan, unpublished); *Xenopus laevis* XL RNA copurified with RNase P [49].

likely reveal the critical structural elements of D-loop mtRNAs that are recognized by the enzyme.

Several structural aspects (including helix C) are similar between all known RNase MRP RNAs and the bacterial and archaeal RNase P RNAs [14–28a]; helix C is also similar between bacterial and eucaryal RNase P RNAs (Fig. 1). While the mammalian RNase MRP enzymes cleave D-loop mtRNA at multiple discrete sites, M1 RNA cleaves specific D-loop mtRNA transcripts at one of the RNase MRP sites [44]. This result is in agreement with the predicted mechanistic conservation between M1 RNA (= the prototype RNase P RNA) and RNase MRP RNAs [14]. However, this result does not exclude the possibility that – depending on the respective RNA–protein interactions within the RNP – certain MRP RNAs may also adopt an alternative structure in the cell [26].

4. PERSPECTIVES

A great deal of progress has been made with regard to the analysis of conserved and unique structural elements in the RNase MRP and RNase P RNAs [14–18,23,26–28a]. However, structural and mechanistic conservation do not predict cellular function. Thus, apart from the requirement for more RNase P/MRP sequences for refined phylogenetic comparisons, three issues require special attention.

(1) What is the function of RNase P/MRP with regard to mitochondrial RNA processing?

Since in many (but not all) cases tRNAs separate the mitochondrially encoded rRNAs and mRNAs and thus 'punctuate' the mitochondrial genome it was suggested that the excision of the tRNAs from the primary transcripts causes the production of both tRNAs themselves as well as rRNAs and mRNAs [45–45b]. This model of symmetric transcription of the mitochondrial genome followed by RNA processing of the polycistronic transcripts by RNase P/MRP-like activities remained unchanged since its proposal [45–45b]. However, the molecular entities required for these processing steps have not been assigned; in fact no activity has been described that processes any mitochondrial precursor RNA at the correct site [45–45b]. It is important to test the 'tRNA excision model' directly, both by rigorous biochemical assays as well as by genetic systems, especially since recent high resolution mapping studies have indicated novel precursors of mature RNA species [46,46a]. These experiments will help to delineate if mechanistic conservation meets functional conservation in ribonuclease P: nuclear encoded (RNP) 'gadgets' may be present in mitochondria in order to participate in the 'removal of 3′-terminal tRNA-like tags, thereby providing functional (primer) RNAs' [43,47].

(2) What is the function of nuclear RNase MRP?

RNase MRP as isolated from nuclear extracts of various eucaryal cell types contained MRP RNA [4,20–22]. Human and plant MRP/7-2 RNA were found in association with higher-order structures in the nucleolus [18]. These results led to the hypothesis that RNase MRP may be involved in RNA processing in the nucleolus [4,12,18,21a].

And finally, (3) What does the association of RNase P/MRP with the Th/To RNP mean for cellular pathways and function?

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